

Review

Induction and function of virus-specific CD4⁺ T cell responsesJason K. Whitmire^{*}

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ARTICLE INFO

Article history:

Received 7 December 2010

Accepted 8 December 2010

Available online 14 January 2011

Keywords:

Virus infection

CD4⁺ T cell memory

T cell activation

Inflammatory cytokines

Interferon

B cells

ABSTRACT

CD4⁺ T cells – often referred to as T-helper cells – play a central role in immune defense and pathogenesis. Virus infections and vaccines stimulate and expand populations of antigen-specific CD4⁺ T cells in mice and in man. These virus-specific CD4⁺ T cells are extremely important in antiviral protection: deficiencies in CD4⁺ T cells are associated with virus reactivation, generalized susceptibility to opportunistic infections, and poor vaccine efficacy. As described below, CD4⁺ T cells influence effector and memory CD8⁺ T cell responses, humoral immunity, and the antimicrobial activity of macrophages and are involved in recruiting cells to sites of infection. This review summarizes a few key points about the dynamics of the CD4⁺ T cell response to virus infection, the positive role of pro-inflammatory cytokines in the differentiation of virus-specific CD4⁺ T cells, and new areas of investigation to improve vaccines against virus infection.

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Introduction

Viruses and the immune system are linked: viruses infect and replicate in an environment where cells are devoted to destroying the infection, so viruses have evolved mechanisms to elude the immune response; conversely, viruses have impacted the survival of their hosts, so the genomes of survivors are imprinted by past battles.

Viruses and the immune system change in real time during an infection with detectable heritable changes in the viral genome and the selective expansion and differentiation of T cells and B cells with epigenetic changes that carry on for the life of the animal. The study of how the immune system copes with virus infection is a key to understanding virus replication strategies and their overall structure, why certain populations of cells are targeted for infection, and the selection of virus mutants with growth advantage. Likewise, one can decipher how the immune system functions by studying how viruses evolve to survive and propagate in hosts.

CD4⁺ T cells impact antiviral immunity at multiple stages of the immune response. CD4⁺ T cells influence antiviral cellular and humoral

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immunity and play a direct role in suppressing virus infection (Fig. 1). As discussed below, CD4+ T cells enhance early expansion of virus-specific primary CTL, their subsequent differentiation into memory cells, guide their localization to sites of infection, and drive secondary expansion of memory CD8+ T cells during re-infection. CD4+ T cells are critical for the processes that lead to long-term humoral immunity, which is the basis of protection for many infections and vaccines. People with deficiencies in CD4+ T cell responses or who have mutations in the molecules involved in CD4–B cell interactions show severe defects in cellular and humoral immunity and suffer from recurrent opportunistic infections. Antiviral CD4+ T cells may interact directly with eosinophils and impact their recruitment and function in lung tissues, potentially contributing to the pathogenesis seen after some lung infections. Thus, virus-specific CD4+ T cells form the nexus between immunologic protection and immune-mediated pathogenesis following infection, so a deep understanding of their induction, regulation, and function is needed to develop safe and effective vaccines against ongoing viral threats. A major point in this review is that CD4+ T cell differentiation and activity is controlled by where they reside.

The T cell response following virus infection consists of several stages (Fig. 2). Innate inflammatory processes begin immediately after infection and are largely driven by various pattern recognition receptors, including toll-like receptors (TLR) that recognize viral material at the cell surface or within the endocytic compartment, and RIG-I-like receptors (RLR) and nucleotide-binding domain-leucine-rich repeat-containing molecules (NLR) that recognize viral material in the cytoplasm. These signals induce type 1 interferon production and other pro-inflammatory cytokines; a cascade of signaling events occurs in cells anywhere near the source of IFN. Interferon stimulated cells are induced to express new proteins that have antiviral activities, including ISG15, ISG20, RNase-L, protein kinase R (PKR), 2',5'oligoadenylate synthetases, Mx1, TRIM5 α , and others (Borrow et al., 2010; Wilkins and Gale, 2010). The effect of IFN can vary by cell type. Besides direct antiviral effects, IFN transiently retains cells in lymphoid tissue by stimulating CD69 expression, which blocks the activity of sphingosine 1-phosphate receptor-1 (Shiow et al., 2006). IFN acts to enhance MHC presentation of antigens to T cells and modifies the proteolytic machinery involved in producing the peptides that will be recognized by T cells. IFNs stimulate APCs to express costimulatory molecules, potentiating the adaptive

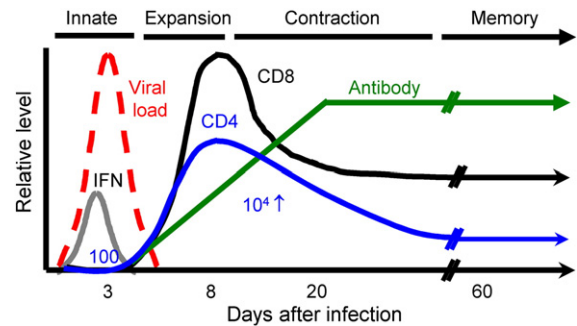


Fig. 2. The T cell response following acute LCMV infection in mice. The T cell response following acute LCMV infection provides a well-defined model to study how CD4+ T cells, CD8+ T cells, and B cell responses develop. The graph shows the dramatic increase in the number of virus-specific T cells after infection and the establishment of elevated numbers of memory T cells. The innate immune response begins immediately after infection and affects the subsequent T cell response. Responding T cells produce interferon-gamma and express other molecules that impact their expansion in number and differentiation into memory cells. The essential patterns are shown for a robust acute infection, but other infections show smaller or delayed responses that are related to differences in the magnitude or duration of infection and variations in the inflammatory environment.

T cell response. During this early time, NK cells become activated and release interferon-gamma. The rapid detection of virus material and production of IFNs serves to diminish virus replication and alert the immune system to infection. Within a few hours after infection, viral antigen is presented to T cells and a few days later, there is an explosive expansion of antiviral T cells (Whitmire et al., 2006, 2008b). It starts from a minute population of cells that begin dividing at incredibly fast rates with the population doubling at a rate on the order of once per 4–5 h, and the responding cells increase in number exponentially during this time (Whitmire et al., 2006). Impressively, as the cells accumulate, they also differentiate into different lineages and transition into memory cells, carry out their direct antiviral functions, travel throughout the body, and assist other wings of the immune system (Whitmire et al., 2006). These events unfold in an inflammatory environment that guides T cell differentiation and is in turn impacted by their presence. The outcome of this complex network of interactions is initially an expanded

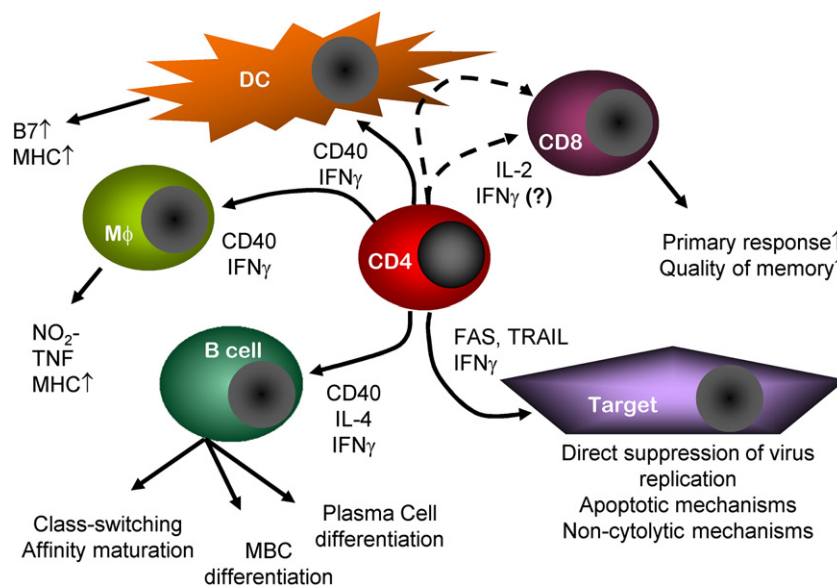


Fig. 1. The central role of virus-specific CD4 T cells in immune defense. Antiviral CD4+ T cells affect multiple wings of the immune system to protect against infection. CD4+ T cells interact directly with antigen presenting cells to enhance their ability to present viral antigen to T cells. They engage B cells and direct their differentiation into memory B cells and plasma cells and affect the kind of antibody that is made. CD4+ T cells can directly suppress virus infection in MHCII+ target cells. CD4+ T cells are essential to induce antiviral CD8+ T cell responses to many infections and enhance the protective recall response of memory CD8+ T cells.

population of short-lived virus-reactive T cells, some of which further differentiate and transition into quiescent memory cells, which confer expedited immunity over extended periods of time. A key point is that innate and adaptive immunity are linked: early inflammatory pathways have an immediate impact in limiting infection and a long-term impact on T cell memory, influencing the kind and number of memory cell that survives and the kind of recall response that develops upon re-infection.

The T cell response following virus infection typically consists of 2 major populations of cells: virus-specific CD8+ T cells and CD4+ T cells

Antiviral CD8+ T cells and CD4+ T cell responses begin from a small number of naïve precursor cells that expand in a non-linear way with roughly similar kinetics of induction and cell-division rate (Whitmire et al., 2006, 2008b). There are a number of differences between CD8+ T cells and CD4+ T cells, which should be considered in order to understand why and how viruses avoid them.

Virus-specific CD8+ T cells and CD4+ T cells recognize different aspects of infection and carry out distinct functions. CD4+ T cells recognize viral antigen that is presented by MHCII molecules on a limited number of cells: dendritic cells (DC), B cells, and monocytes/macrophages. CD4+ T cells “see” fragments of extracellular material that professional antigen presenting cells (APC) have taken up, degraded, and presented in the context of MHCII. DCs efficiently take-up antigen in the periphery, then travel to the draining lymph nodes, where they process and present antigen to T cells. The separation between scavenging for foreign material and the presentation of that material at different sites is seen for inert antigens, but may or may not hold following live virus infections where there is active recruitment of activated APCs to the site of infection. As discussed below, CD4+ T cells can engage these cells out in peripheral sites and impact their behavior or localization. The extracellular material can include viral particles, viral proteins, and viral debris from necrotic cells. In contrast to CD4+ T cells, CD8+ T cells recognize MHC1 molecules that are loaded with peptides derived from intracellular proteins; these proteins are ubiquitinated and degraded by the proteasome or immunoproteasome into peptides that are shuttled into the secretory pathway, where they are loaded onto MHC1 molecules. The epitopes that are presented on MHC1 molecules are largely derived from viral protein that has entered the cytosol as a result of infection (fusion) or from proteins arising from the *de novo* synthesis of viral protein from viral nucleic acid sequences. CD8+ T cells can be stimulated by extracellular protein through a process termed “cross-presentation”, but the protective value of this presentation, and the extent to which cross-presentation happens during the course of a natural infection is not clear and could be somewhat less efficient compared with endogenous “classical” pathways of antigen presentation; a recent study of vaccinia virus infection found that direct presentation, and not cross-presentation, plays the major role in the induction of antiviral CD8+ T cells (Xu et al., 2010). There are several recent reviews of cross-presentation (Bevan, 2006; Blanchard and Shastri, 2010; Rock et al., 2010; Yewdell, 2010; Yewdell and Haeryfar, 2005).

CD4+ T cells can be induced by cells that are not actually infected and recognize antigen from non-replicating virus material, so they can be far more sensitive to foreign antigen than CD8+ T cells. For example, non-replicating or inactivated vaccines composed of viral proteins or bacterial toxins induce CD4+ T cell responses but negligible CD8+ T cell responses. Many viruses effectively inhibit antigen-presentation to CD8+ T cells, but such blockade does not affect CD4+ T cells, since non-infected cells still efficiently take up and present antigen. An example of this effect is seen following Coxsackie virus infection of mice; certain viral proteins (2B, 2BC, 3A) prevent the surface expression of MHC1 (Cornell et al., 2006, 2007). Minimal to no detectable CD8 response is induced in these mice, even when using extremely sensitive techniques; in contrast, CVB3-specific

CD4+ T cells are stimulated and readily measured (Kemball et al., 2008, 2009). Other viruses, such as the myxoma virus (Zuniga et al., 1999), adenovirus (Blair and Blair-Zajdel, 2004; Windheim et al., 2004), HSV (Ahn et al., 1996; Barcy and Corey, 2001; Neumann et al., 2003; Orr et al., 2005; Sievers et al., 2002; Temme et al., 2010), Varicella-zoster virus (Eisfeld et al., 2007), MCMV (del Val et al., 1992; Heise et al., 1998; Lemmermann et al., 2010), and HCMV (Jackson et al., in press), also have elaborate mechanisms to prevent MHC antigen presentation, thus reducing their visibility to the immune system. It is intrinsically difficult for viruses to dodge CD4+ T cell responses, but several major mechanisms have been identified, including the production of viral homologs of IL-10 (EBV, MCMV) (Kanai et al., 2007; Knappe et al., 2000; Kutenko et al., 2000) to generally suppress MHC expression, targeting DC for depletion by CD8+ T cells (Zuniga et al., 2008), and down-regulating MHCII expression on infected cells (Hegde et al., 2002; Lewandowski et al., 1993).

Another key difference between virus-specific CD8+ T cells and CD4+ T cells is the efficiency with which they kill target cells: this is done expeditiously by antigen-experienced CD8+ T cells, whereas such killing by CD4+ T cells is sluggish. This can be demonstrated using an “in vivo CTL assay”. In this assay, target cells are loaded with specific or irrelevant peptides and then differentially labeled with a dye (CFSE) and intravenously transferred into the same host; after a short period of time, flow cytometry is used to compare the relative loss of the specific peptide-coated cells compared to the control target cells. When target cells are loaded with an LCMV peptide that binds MHC1 and is recognized by immunodominant LCMV-specific CD8+ T cells, they are completely eliminated within minutes upon transfer into an LCMV-immune mouse. By comparison, only a small fraction of GP_{61–80}-loaded target cells are killed in these mice over a time span of one day. Thus cytolytic CD4+ T cells emerge after infection (Jellison et al., 2005), but their protective role based on direct killing of target cells after infection pales in comparison with virus-specific CD8+ T cells that kill within minutes (Yates et al., 2007). This is underscored by the inability of CD8-deficient mice to resolve LCMV infection despite the presence of large numbers of virus-specific CD4+ T cells. The mechanisms of killing are also very different: CD8+ CTL rely upon preformed perforin and granzyme molecules to poke holes into the target cells; CD4+ T cells utilize FAS-L and TRAIL to induce a caspase-dependent apoptosis. The dichotomy in rapid killing ability makes sense given that cytolytic cells are restricted to targets that are actually infected as opposed to nearby antigen presenting cells that have taken up antigen and are involved in alerting the immune system to infection. Such restricted killing may be beneficial by preserving APC whose depletion would cause generalized immune incompetence. An example of the detrimental effects of APC loss are seen following infection of mice with LCMV variants that selectively infect DC; the resulting CTL response eliminates these cells and the mice become susceptible to other infections (Sevilla et al., 2000, 2003; Zuniga et al., 2008). Similar pathogenesis may occur in people exposed to influenza, who then acquire lethal bacterial infections. Virus-specific CD4+ and CD8+ T cells share the ability to directly suppress virus replication by bathing target cells in IFN γ , but CD4+ T cells accomplish this without the collateral damage caused by perforin-dependent cell killing.

CD4+ T cells help CD8+ T cell-dependent antiviral defenses

For many virus infections CD4+ T cells are needed to drive the initial expansion of virus-specific CD8+ T cells (Williams and Bevan, 2007). In the absence of CD4+ T cells there is minimal expansion of CD8+ T cells and deficient cellular immunity. Virus-specific CD4+ T cells engage DCs that are shared with reactive CD8+ T cells; the CD4+ T cells stimulate the APCs to express costimulatory molecules and to increase the presentation of viral antigen so that the APCs better stimulate CD8+ T cells. CD4+ T cells are efficient producers of IL-2, which is a growth factor for CD8+ T cells and encourages improved cell-division and cell

survival. It is plausible that virus-specific CD4+ T cell production of IL-2 (and IFN γ , see below) acts on nearby virus-specific CD8+ T cells. Such interactions might occur when both cells are adjacent to a virus infected APC.

Many virus infections grow to high titer in vivo and, due to efficient MHC I presentation, readily stimulate virus-specific CD8+ T cells. Virus-specific CD4+ T cells often are not needed for the initial expansion of CD8+ T cells in these models. However, CD4+ T cells affect the subsequent ability of memory CD8+ T cells to undergo vigorous secondary responses upon re-challenge with infection (Hamilton et al., 2006; Janssen et al., 2003; Shedlock and Shen, 2003; Sun and Bevan, 2003; Sun et al., 2004). Evidence indicates that early CD4-dependent signals leave a lasting effect on memory cell formation; help-less CD8 T cells expand poorly compared to those that differentiate in the presence of CD4+ T cells. Other evidence implicates memory CD4+ T cell effects during the actual recall response itself (Agnellini et al., 2008). In this case, CD4+ T cells deliver signals – and it is not clear what they are – that further the ability of responding memory CD8+ T cells to accumulate in number (Sun et al., 2004). The simultaneous activation of memory CD4+ T cells and memory CD8+ T cells results in a more protective CTL response compared to the activation of CD8+ T cells alone.

Antiviral T cells circulate widely after infection (Marshall et al., 2001; Masopust et al., 2001; Whitmire et al., 2006). In several model systems, antiviral CD4+ T cells are needed to recruit CD8+ T cells and other cell types to peripheral sites of infection. For example, CD4+ T cells recruit mouse hepatitis virus-specific CD8+ T cells into the CNS (Stohlman et al., 1998). CD4+ T cells recruit influenza-specific CD8+ T cells into the lung (Teijaro et al., 2010). HSV2-specific CD4+ T cells enhance CD8+ T cell movement to HSV2 infected tissues, and the effect is dependent upon IFN γ production by the CD4+ T cells and the local induction of CXCR3 that allows circulating CD8+ T cells to enter the tissue (Nakanishi et al., 2009). Perhaps the local production of IFN $\alpha\beta$ caused by infection results in an influx of cells, including those with antigen presenting functions and CD4+ T cells. Once there, CD4+ T cells direct other responses locally and guide other cell types into the infected tissue (Lane et al., 2000).

Virus-specific CD4+ T cells sustain antiviral CD8+ T cell responses during protracted infections (Khanolkar et al., 2004; Kumaraguru et al., 2005; Wherry et al., 2007; Zajac et al., 1998a). For example, variants of LCMV will grow to high titer and target new populations of cells and disseminate throughout the body of the mouse. The CD8+ T cell response against these variants is strained and eventually virus-specific CD8+ T cells either undergo apoptosis or become dysfunctional (exhausted), losing their ability to proliferate and produce cytokines when exposed to their cognate antigen. Eventually, wildtype mice reduce the viral load to levels at or below the detection limits of plaque assay in most tissues but not mice that are depleted of CD4+ T cells (Battegay et al., 1994; Leist et al., 1989; Matloubian et al., 1994). Transient depletion of CD4+ T cells is sufficient to prevent long-term control of these variants. In wildtype mice, functional cytolytic CD8+ T cells can be recovered once the infection is controlled, but in CD4-deficient mice, functional CD8+ T cells are not recovered. Dysfunctional CD8+ T cells can be rescued by the adoptive transfer of functional CD4+ T cells (Homann et al., 1998; Kumaraguru et al., 2005). The mechanism by which CD4+ T cells rescue virus-specific CD8+ T cells is elusive; it could be related to the release of copious amounts of IL-2 that acts on CD8+ T cells, the ability of CD4+ T cells to stimulate APC activity or survival that indirectly sustains CD8+ T cells, or possibly modulation of inhibitory receptor–ligand interactions between CD8+ T cells and APCs. Virus-specific CD4+ T cell production of IL-21 sustains antiviral CD8+ T cells during persistent virus infection (Elsaesser et al., 2009; Frohlich et al., 2009; Melief and Schoenberger, 2010; Yi et al., 2009, 2010). CD4+ T cells may also protect APCs from CTL-mediated killing (Mueller et al., 2006), thus preserving their immune-stimulatory functions.

Big responses from small numbers

The T cell Receptor consists of several subunits, and the regions involved in binding peptide and MHC complexes are highly variable and lead to a diverse array of pro-T cells that travel from the bone marrow to the thymus; a subset of the cells go on to differentiate into single positive CD4+ or CD8+ T cells that populate the peripheral lymphoid organs. Based on the number of domains, their random juxtaposition and additional sequences that are added at the joining sites, the theoretical variety of unique TCRs is enormous and far greater than the actual number of cells in a mouse. Until recently, the frequency of any given antigen-specific T cell in the naïve pool was unknown, but it was estimated by CDR3-length measurements of different classes of TCR molecules that there are approximately 2×10^6 distinct T cell precursor pools, each containing an average of 10 cells (Casrouge et al., 2000). This estimate was largely borne-out by direct measurements of antigen-specific cells using either functional methods or tetramer-enrichment techniques. For example, in an uninfected wildtype (B6) mouse, there are approximately 100 CD4+ T cells with the potential to respond to an immuno-dominant I-Ab-restricted epitope of LCMV (GP_{61–80}) (MacLeod et al., 2008; Whitmire et al., 2006). A similar frequency for an immuno-dominant LCMV-specific CD8+ T cell population (Blattman et al., 2002) indicates that there is overlap in the frequencies of precursors between immuno-dominant CD4+ and CD8+ T cells. The frequency for other epitope-specific T cell precursors ranges from ~10–1000 per mouse (Hataye et al., 2006; Moon et al., 2007; Obar et al., 2008). There is a trend where the initial naïve precursor frequency correlates with the subsequent immunodominance hierarchy that is seen following infection, but this pattern does not always hold.

The induction phase of the response commences immediately upon infection and involves viral antigen along with inflammatory signals and costimulatory signals. Within a few hours after infection, viral antigen is presented to T cells at sufficient levels to stimulate antigen-experienced cells to make cytokine (Whitmire et al., 2008b). This can be shown experimentally by re-challenging immune mice, which contain large numbers of memory T cells, and then directly injecting BFA soon afterwards. The BFA acts to prevent T cell secretion of IFN γ , so reactive cells accumulate this cytokine following antigen exposure in vivo. The cells are isolated and immediately stained for intracellular IFN γ without further ex-vivo manipulation. A large fraction of virus-specific CD4+ T cells will express IFN γ 6–12 h after infection, indicating that those cells recognized their cognate antigen in vivo. The actual speed with which antigen is presented may be much faster and on a timescale of minutes. From the standpoint of virus infection, it is important to have an adaptive response that matches the rate at which viruses replicate and spread. These events can happen quickly (such as LCMV replication and spread in mice) and the T cell response occurs quickly; other infections are slow and the resulting T cell response dithers. For example, the T cell responses against mycobacteria peak one month after infection; the T cell responses against the hepatotropic viruses HAV and HCV only emerge 4–6 weeks after infection. It is unclear why the kinetics vary so dramatically—different viruses and other pathogens influence the kind of milieu that forms during these critical stages of the T cell response, and this may explain the variation in kinetics, quality and magnitude of subsequent memory T cells. It is also possible that some viruses have evolved mechanisms to delay the kinetics to facilitate virus survival and spread. For example, the liver environment suppresses immune responses, which may explain why viruses targeting that location induce small and delayed immune responses.

Despite the initial presence of LCMV antigen, virus-specific naïve and memory T cells undergo cell division after a delay of up to 3 days, followed by an explosive transition into extremely rapid cell division and accumulation (Whitmire et al., 2008b). The initiation of the response is governed, at least in part, by the inflammatory environment as opposed to intrinsic limits governing the ability of T cells to transform

from quiescent cells to ones that are fully capable of dividing. T cells that are placed within an inflamed environment (i.e. a pre-infected mouse) are able to begin dividing without much delay (2 days as opposed to 3–4 days), which implies that certain signals beyond antigen, are involved in initiating the rapid response. These signals could include the induction of positive regulators or the elimination of negative regulators or Treg cells.

As the response develops, populations of cells are differentially impacted by various selective forces – including antigen abundance, TCR-peptide/MHC affinity, and cytokines – that determine the cells that preferentially grow out in number and contribute to T cell differentiation and survival into the memory phase. The magnitude of the CD4 response is largely governed by the amount and duration of the antigen (Obst et al., 2005; Ravkov and Williams, 2009). The CD4 response truncates when infection is artificially terminated, such as by treating recombinant-*Listeria monocytogenes*-infected mice with antibiotics. Similar findings were seen following recall responses. These analyses were done in the context of LM-driven T cell responses; whether similar correlations hold for virus infection remains to be shown. Analogous experiments using ribavirin in virus infected mice should be done to evaluate whether antiviral CD4+ T cell responses are also highly dependent on antigen-dose and duration.

Remarkably, much of the virus-specific CD4+ T cell accumulation occurs within a few days, implying cell-division rates on the order of one population doubling every 4–5 h, but possibly faster if some of the daughter cells undergo apoptosis during the response. Given the speed with which viruses replicate, it is important to have an adaptive immune system that can keep up; on the other hand, a robust autoreactive response or one that is otherwise misdirected could lead to catastrophic immune-mediated pathology. Therefore, the response is heavily governed, but the details of how this occurs are obscure.

Virus infections stimulate distinct lineages of CD4+ T cells that serve unique functions

As antigen-specific CD4+ T cells respond to infection, they differentiate into lineages and produce characteristic sets of cytokines that impact how they influence other components of the immune response. They also express certain chemokine receptors that govern whether they travel to peripheral sites, localize within lymphoid organs in T cell zones, or localize near B cell zones. The overall kinetics of the CD4+ T cell response and their survival/maintenance into the memory phase has been studied in models that induce predominantly a Th1-type response. Antiviral Th1 cells are commonly found after live virus infections, because target cells typically produce IFN $\alpha\beta$, and DC and NK cells produce IL-12, IL-18 and IFN γ that stimulate cells to differentiate into the Th1 path. Th1 cells are located in lymphoid organs but also travel to peripheral sites. They express IFN γ , which acts on target cells to directly suppress virus replication and augments antigen presentation by DC, B cells, and macrophages. Th1 cells also make IL-2 to support the expansion of other responding T cells. Th1 cells in the periphery produce IFN γ that modulates chemokine gradients and causes macrophages and CD8+ T cells to be recruited to the sites of infection.

Virus-specific T_{FH} cells emerge after infection (Johnston et al., 2009; Yusuf et al., 2010). T_{FH} cells preferentially localize to the B cell boundaries within lymph nodes rather than circulate to peripheral sites (McHeyzer-Williams and McHeyzer-Williams, 2004). Given their proximal location to B cell areas, it is thought that T_{FH} cells are directly involved in B cell differentiation, including the germinal center response and memory B cell formation (McHeyzer-Williams et al., 2009). These CD4+ T cells express CD40L and SAP that are vital for stimulating virus-specific B cells to further differentiate into MBC or plasma cells (Crotty et al., 2003; McHeyzer-Williams and McHeyzer-Williams, 2004). Virus-specific T_{FH} cells express IL-4 (a B cell growth factor and differentiation factor), IFN γ (also a B cell differentiation factor), and CD40L. The ability of

T_{FH} cells to simultaneously make IFN γ and IL-4 may explain why IgG1 and IgG2a antibodies are often found together after infection. Many interesting questions remain about this population of cells: are they short-lived or long-lived; do they form memory; how are they related to other populations of virus-specific CD4+ T cells.

The patterns and behaviors of the other lineages may be similar or different to Th1 cells, so comparable analyses in other infection models would be useful, as they would provide insights about adjuvant design and overall regulation of immunity and immune-mediated pathogenesis. For example, virus-specific Th2 cells are found after some infections. RSV in particular induces a Th2 response that is associated with pathogenesis and an excessive influx of eosinophils into the lung. Virus-specific Th₁₇, Th₂₁, Th₉, and T_{Reg} emerge after virus infections, but much remains to be learned about their regulation, function, and longevity. Virus-specific cytotoxic CD4+ T cells are found during some virus infections (HCMV, MCMV, others) (Appay et al., 2002). Cytotoxic CD4+ T cells express IFN γ and granzyme and FasL could be regarded as a subset of Th1 cells, but it is possible that they are a unique lineage, and their relationship to other cells is not clear. These cells hold potential for therapeutic applications: when primed and expanded artificially, they can be adoptively transferred into people with certain tumors to kill transformed cells.

It is important to consider that live virus infections create unique milieus that result in complex mixtures of responses, so in an individual there can be a mixture of CD4+ T cells from different lineages (Marzo et al., 2002; Roman et al., 2002). CD4+ T cells tend to fall neatly into distinct lineages, but recent data suggest there is pliability: Th2 cells can be “re-programmed” into Th1 when exposed to the interferons that are expressed during virus infection (Hegazy et al., 2010); other data indicate that IFN γ – the proto-typical Th1-inducing cytokine – is also important for Th2 cell formation (Bocek et al., 2004). The environment in which the virus grows changes with time, so it is plausible that CD4 differentiation is flexible and varies according to the local abundances of cytokines and antigen that occur throughout the response.

Memory T cells are a first line of defense against re-infection

CD4 T cell memory is typically seen after infection or vaccination and, where examined, it is long-lived. There are some examples of memory waning over time (Homann et al., 2001), but other data show fairly stable levels over time (Hammarlund et al., 2003; Harrington et al., 2002; Varga and Welsh, 1998; Whitmire et al., 1998). Evidence from studies utilizing “marked effector cells” indicate that memory T cells are derived from antigen-experienced effector cells in a linear differentiation process from naïve to effector to memory cell (Harrington et al., 2008; Hu et al., 2001; Jacob and Baltimore, 1999; Opferman et al., 1999). These studies used transgenic T cells that contain an inactive marker, which recombines when the T cells are stimulated by antigen; those T cells and all of their progeny can be identified by flow cytometry. Several studies using this approach show that memory cells that form after infection are marked, indicating a direct relationship between the effector cells and memory cells.

Memory precursors can be identified early on based on their expression of IL-7R and their ability to bind tetramers (Kaech et al., 2003; Kondrack et al., 2003; Lenz et al., 2004; Li et al., 2003; Seddon et al., 2003). During the initial response memory precursors emerge. Virus-specific CD4+ T cells down-regulate IL-7R, but there are cells that re-express IL-7R (Kaech et al., 2003; Kondrack et al., 2003; Lenz et al., 2004; Li et al., 2003; Seddon et al., 2003) and down-regulate KLRG1 (Hand et al., 2007; Joshi et al., 2007). After the infection is resolved, the swollen number of effector cells is no longer needed and it is energetically expensive to sustain such a vast population of cells. Therefore, most of the cells (~90%) are purged during the contraction phase and the survivors have the characteristics of memory cells (described below).

The other effector cells undergo cell death and the IL-7R+ population accumulates (or at least doesn't die); over time, only IL-7R+ cells remain and they behave like memory cells. The increased proportion of IL-7R+ cells that is seen during the contraction phase could either be the result of deletion of IL-7R- cells, an intrinsic change in cells from IL-7R- to IL-7R+, or a combination of IL-7R+ cell division and accumulation along with the loss of IL-7R- cells; there is evidence consistent with all of these scenarios. The purging process is enigmatic because cell selection and survival into the memory phase occurs in the apparent absence of virus replication and inflammation. Most evidence indicates that the virus-specific T cells undergo caspase-dependent apoptosis, and the surviving T cells tend to express increased amounts of Bcl2, Bcl-xL, and Bcl6 (Grayson et al., 2000, 2006; Homann et al., 2001; Pipkin et al., 2010) compared to effector cells.

Once established, CD4 T cell memory does not require antigen (Swain et al., 1999) but depends upon cytokines derived from stromal cells. Antiviral CD4+ T cells use IL-7 and, to a lower extent, IL-15 for homeostatic maintenance (Jaleco et al., 2003; Lenz et al., 2004; Purton et al., 2007). In the absence of these cytokines, virus-specific CD4+ T cells decline in number; conversely, their number can be increased above normal when recombinant versions of these cytokines are given to mice (Boyman et al., 2006b, 2008; Cho et al., 2007; Rubinstein et al., 2006).

Quiescent memory T cells are responsible for the expedited immunologic protection seen after immunization. As described below, naïve and memory cells differ dramatically in frequency, sensitivity to antigen, cytokine output, and location, which explain why memory cells confer enhanced immunity compared to naïve cells.

Frequency

Immune mice mount recall responses that are detectable sooner than primary responses. The accelerated appearance of the recall response can be explained by the differences in frequency between virus-specific naïve cells (~100 per mouse) that are well below detection by standard assays versus memory cells that can be 500–1000-fold more abundant. Upon challenge, the naïve cells need to undergo far more rounds of cell division and accumulation before they are detected; memory T cells only need to go through a few rounds; hence, memory responses appear sooner. Most analyses of memory T cells have been done in immune mice, and the results from these mice were compared to those from naïve mice. However, there are numerous differences between these two groups of mice, including the virus-specific T cell frequency and the presence of virus-specific antibody and CTL that affect antigen load and APC function. To investigate whether the differentiation changes that occur during the transition of effector cells into memory cells result in quicker cell division rates, LCMV-specific T cells were transferred into naïve mice that were subsequently infected (Whitmire et al., 2008b) and were allowed to differentiate into memory T cells. These memory T cells were isolated, mixed with naïve cells, labeled with CFSE, and adoptively transferred into the same naïve host. Upon infection, the two donor populations were identified by flow cytometry. The data indicate that when compared in the same host and at similar frequencies, memory T cells and naïve T cells begin cell-division at around the same time (Whitmire et al., 2008b).

The recall response mediated by memory T cells is typically more robust than the primary response. Once naïve and memory T cells begin dividing, the effector cells derived from the memory T cells accumulate more efficiently than the primary effector cells (MacLeod et al., 2008; Whitmire et al., 2008b). As a result, there is a rapid rise in the number of secondary effectors. The rapid accumulation of memory cells compared to their naïve counterparts may relate to the efficiency with which memory cells produce cytokines (IFN γ , IL-2) that increase cell division or prevent apoptosis; however, the accumulation could be

the result of other epigenetic changes (intrinsically elevated Bcl2 levels). There may also be refinement of virus-specific cells so that only the best responders are selected to survive into the memory phase; these cells may have slight alterations in TCR structure that make them better able to respond compared to the mixed population of naïve T cells that react to the same epitope (Williams et al., 2008). Other genomic changes may be at play in enhancing the responsiveness of memory cells following infection, including their increased frequency in peripheral tissues (Klonowski et al., 2004; Lefrançois, 2006).

Sensitivity to antigen

Compared to naïve cells, memory T cells are 50–500-fold more sensitive to antigen (Slifka and Whitton, 2001; Whitmire et al., 2006; Williams et al., 2008). The improvement in sensitivity to low quantities of antigen occurs during the expansion phase and carries on into memory. The molecular mechanisms that permit greater sensitivity have not been worked out: there may be differences in TCR abundance, preformed signaling components associated with the TCR, or increased levels of accessory molecules that improve T cell association with target cells. For example, memory cells express pre-formed costimulatory molecules that can immediately be secreted to the surface of cells to engage APCs (Koguchi et al., 2007).

Effector functions

A major difference between memory T cells and naïve T cells is their ability to rapidly make cytokine (Liu et al., 2004; Rogers et al., 2000). When exposed to infection, memory T cells secrete IFN γ within minutes. We utilized an in-vivo method to detect T cells that make IFN γ after infection (Foster et al., 2007; Liu and Whitton, 2005; Whitmire et al., 2008b). The method involves infecting mice and then treating them with Brefeldin-A to force reactive T cells to sequester cytokine intracellularly. Splenocytes are harvested and immediately surface stained to identify virus-specific cells and stained for intracellular levels of IFN γ . Using this assay, we compared naïve and memory T cells in the same host and found that memory cells respond to infection by producing IFN γ by 6–12 h after infection; naïve T cells in the same conditions did not make detectable levels of IFN γ . (Whitmire et al., 2008b) These data show that memory cells are geared to respond quickly to infection by producing antiviral cytokine. The IFN γ locus is epigenetically modified in memory cells compared to naïve cells (Kersh et al., 2006; Northrop et al., 2006); the histones at this locus are demethylated, thus making the local heterochromatin open and accessible to transcription factors. These data resemble what is found for memory CD8+ T cells: virus-specific CD8+ T cells in immune mice kill target cells within minutes (Barber et al., 2003; Yates et al., 2007). Hence, a quiescent population of CD8+ memory cells can defend very quickly and during the same periods usually associated only with the innate immune system—thus, memory T cells exert their effects long before their accumulation appears.

Cytokine patterns

As responding T cells differentiate, they increase their ability to produce IFN γ , TNF, and IL-2. It is not clear why there is a difference in kinetics between IFN γ production and IL-2 secretion, but it may relate to the antiviral activity of IFN γ , which is needed immediately. The ability to make these cytokines appears to be sequential: early on many cells make IFN γ but not IL-2, but by the peak of the response, most effector cells make all three cytokines. The early production of IFN γ and TNF serves to diminish virus infection and activate macrophages and dendritic cells. IFN γ production by responding T cells may be directly linked to their enhanced expansion (see below). Early production of IL-2 could be detrimental to expanding effector cells if its impact on NK cells or Treg cells is greater than that

on T cells. The delayed production of IL-2, a growth and survival factor of T cells, may correspond with the transition of effector cells into the memory phase: IL-2 given during the contraction protects antiviral CD8+ and CD4+ T cells from apoptosis and increases memory. The expedited production of IL-2 by memory T cells may play a role in accelerating their accumulation once cell-division commences.

Interferons simultaneously suppress virus replication in target cells and stimulate antiviral T cells that ward off further infection

Numerous viruses encode proteins that either prevent the induction of IFN or block signaling cascades that result from IFN signals. RNA viruses are usually capped to prevent recognition by PRRs and induction of interferon; additionally, members of the picornavirus family (CVB, poliovirus, HAV) prevent encode proteins that block parts of the interferon induction process or block interferon-receptor signals. Other RNA viruses, such as members of the flavivirus produce non-structural proteins that prevent type 1 IFN induction as well as IFN signaling. Vaccinia virus encodes decoy receptors for both type 1 and type 2 interferons. The elaborate mechanisms that viruses use to inhibit IFN highlight the key role of these pathways in immune defense.

Numerous pathways are involved in the inflammatory response and result in signals that act directly on T cells. For example, PRRs (NLR, TLR) are involved in initiating the response and lead to IFN $\alpha\beta$ production by the infected cell, which may act directly on responding T cells and impact their functions or accumulation. Also, early IFN γ production by NK cells and responding T cells acts upon the IFN γ R, which leads to JAK1/2 recruitment followed by STAT1 phosphorylation by JAK1. The STAT1-PO4 forms homodimers that travel to the nucleus and bind to promoter elements called Gamma Activated Sequences, which are near hundreds of genes that are upregulated as a consequence of IFN γ R signaling. IFN functions immediately during infection, but the long-term consequence of IFN signaling is the stimulation large numbers of effector and memory T cells. The expansion of the CD4+ T cell population depends on several critical pathways, which likely influences the timing and magnitude of T cell responses. Sequential waves of interferons and costimulatory molecules are expressed after infection, and these interactions may result in distinct effects on T cell differentiation that varies with time (Bertram et al., 2002).

We have shown that T cells need direct IFN γ signals to fully expand, and they compete for this cytokine as they differentiate into effectors and memory cells (Whitmire et al., 2005a,b, 2007, 2008a). The receptor for IFN γ , CD119, is expressed ubiquitously; however, the surface level of expression increases selectively on responding virus-specific T cells, consistent with the notion that IFN signals directly into T cells and enhances their ability to respond. To better understand the role of direct IFN signals in virus-specific T cell differentiation, we compared in the same host T cells that were genetically deficient in γ R-expression with T cells that express γ R (Whitmire et al., 2005a,b, 2007, 2008a). Under these conditions, the T cells – which are identical in their specificity – were transferred into the same WT host, where they were exposed to the same antigen load and duration. We found that after infection, γ R– T cells expanded to lower numbers compared to the T cells that expressed γ R, despite being present at equal numbers before infection. Similar results were seen when comparing γ R+ versus γ R– TCR-transgenic T cells or when endogenous virus-specific T cells from γ R+ or γ R– mice were compared in the same host. Our findings closely parallel those reported for IFN $\alpha\beta$ signals, where T cells that are sensitive to IFN $\alpha\beta$ expand in number more vigorously than do T cells that are $\alpha\beta$ R-deficient (Havenar-Daughton et al., 2006; Kolumam et al., 2005), and we confirmed this observation (unpublished data). The findings with $\alpha\beta$ R-deficient T cells are consistent with the evidence that TLRs enhance T cell memory; TLR signals alter APC antigen presentation capacity and stimulate IFN $\alpha\beta$ production from them, which acts directly on responding T cells. Thus, IFNs act on target cells to diminish virus

replication and act on responding virus-specific T cells to augment their accumulation.

Some reports indicate that IFNs are detrimental to T cell memory formation or that there is no impact on memory cell formation (Badovinac et al., 2004; Dalton et al., 2000; Haring and Harty, 2006; Tewari et al., 2007). The varied conclusions likely stem from differences in the methods and model systems. For example, analyses comparing T cell memory in separate WT and IFN-deficient mice is problematic when the pathogen load and duration differs. A similar concern holds when a large proportion of APCs or other cell-types (T-reg cells, etc.) are IFN γ R-deficient, as in a bone-marrow chimera. It is important to consider that the role of IFN likely varies depending on the pathogen: virus infections typically stimulate large amounts of IFN, but bacterial infections stimulate an entirely different set of cytokines; other cytokine networks may dominate in stimulating T cell responses after bacterial infection. The effect of direct IFN signals appears after day 4 post-LCMV infection, when the cells are accumulating exponentially; the role of IFN may not be as apparent in infections or vaccinations where the stimulus does not drive extensive cell division and accumulation. Hence, weak stimuli that drive only a few fold changes in cell number may not appear to be impacted by IFNs.

During a response, restrictive numbers of APC force T cells to compete for antigen; under increasing amounts of competition, smaller and smaller proportions of the cells undergo cell division. We have shown, by varying the frequency of naïve virus-specific TCR-transgenic T cells before infection, that an overabundance of identical T cells is detrimental to antiviral T cell memory. Virus-specific CD8+ T cells and other CD4+ T cell responses show a similar pattern (Blair and Lefrancois, 2007; Foulds and Shen, 2006; Hataye et al., 2006; Kedl et al., 2000; Sarkar et al., 2007; Srinivasan et al., 2004; Troy and Shen, 2003; Whitmire et al., 2008a; Willis et al., 2006). The negative impact on T cell memory suggests that T cell differentiation may be linked to cell division. As antigen becomes limiting and T cell number increases, the competition for antigen becomes severe with time. T cell production of IFN γ is tightly controlled by contact with antigen (Slifka et al., 1999), so the effect of constrained amounts of antigen impacts virus-specific T cell production of IFN γ , thus limiting direct IFN γ signaling into T cells.

We propose a link between T cells that most efficiently elaborate IFN γ secretion and receive IFN γ R signals and their competitive fitness to out-expand other T cells and go on to differentiate into memory T cells following acute virus infection (Fig. 3). IFNs in particular select cells into the memory pool: competition between cells for limiting amounts of IFN is a deciding factor in determining which cells survive initial expansion and go on to populate the memory pool. It is not yet clear whether the effect of IFN γ R signals merely increases the abundance of cells with the potential to differentiate into memory cells or directly stimulates the memory program. However, it is noteworthy that in the absence of direct γ R signals, there is a precipitous loss of T cell memory compared to identical cells that express γ R. In other words, the initial difference between the two populations at the peak of the response exaggerates as the cells differentiate into the memory phase, suggesting that there is a direct connection between these signals and the memory program. Further studies are required to delineate how direct IFN signals impact memory cell formation and if the long-term effects are set early on or actively deployed during the contraction phase.

T cells also appear to compete for limiting amounts of IL-7, IL-15, and IL-2: when these cytokines are delivered as complexes to mice so that they are overabundant across several days, antigen-specific T cells are driven to tremendous number (Boyman et al., 2006a,b, 2008; Finkelman et al., 1993; Kamimura and Bevan, 2007; Kamimura et al., 2006; Purton et al., 2008; Rubinstein et al., 2006). Conversely, when T cells are starved of these cytokines, there are severe reductions in the initial expansion and memory levels. It is plausible that these cytokines factor into the memory selection process and support translational efforts to use

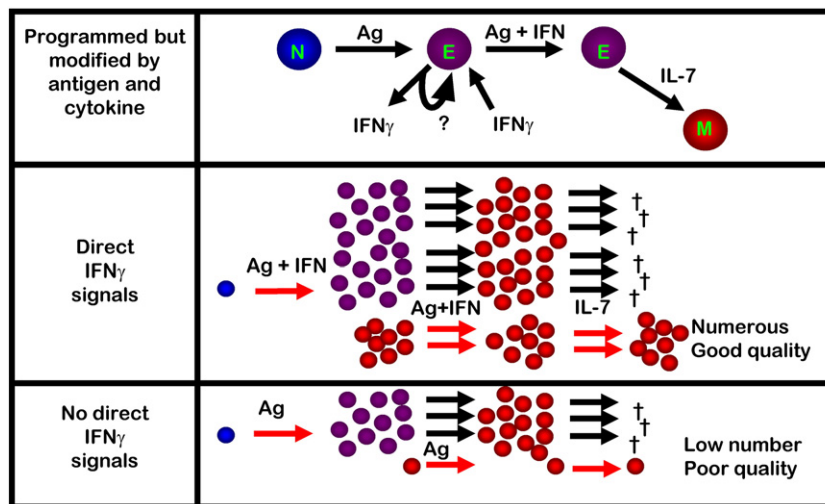


Fig. 3. Selection of the fittest: virus-specific CD4⁺ T cells compete for antigen and cytokines, selecting and enriching for high quality memory cells. Antiviral CD4⁺ T cells respond to antigen and inflammation by undergoing cell-division, which can be incredibly rapid. As they accumulate in number, effector T cells begin to compete for access to antigen, which constrains cell-division for cells that weakly interact with antigen. In addition, the most vigorously responding T cells produce IFN γ and receive direct IFN γ signals; the best responders receive the greatest amount of IFN γ signaling and continue to accumulate. IFN γ signals also enhance memory cell formation. It is unclear if direct IFN γ signals activate a program of memory cell differentiation or increase the number of memory cell precursors that have the potential to survive.

cytokine-mediated immunotherapy to increase the abundance of vaccine-induced memory T cells.

There are gaps in our understanding of how other pro-inflammatory pathways affect T cell memory formation. Some pathways act directly on responding T cells, others function indirectly through APCs, and some (MyD88, IFN $\alpha\beta$, IFN γ) act on both. NLRs sense bacterial and some DNA or RNA virus infections (Allen et al., 2009; Ichinohe et al., 2009; Kuenzel et al., 2010; Lamkanfi and Kanneganti, 2010; Neerincx et al., 2010). NLRs induce IL-1 β production, which signals directly into T cells to enhance their expansion (Ben-Sasson et al., 2009). Consistent with this notion, IL-1R-deficient mice show deficits in lung CD4⁺ T cell responses following influenza infection (Schmitz et al., 2005). IL-12 and IL-18 are made by activated dendritic cells after virus infection; T cells express receptors for these cytokines and their differentiation into Th1 cells is impacted by these signals (Srinivasan et al., 2007) and may affect the expansion of CD4⁺ T cells and their differentiation into memory cells (Boelen et al., 2002; Haring and Harty, 2009; Pien et al., 2000; Srinivasan et al., 2007; Tough et al., 2001). Type-III interferons (IFN λ) are produced during some virus infections and are particularly abundant at skin and mucosal sites. IFN λ is stimulated by some of the pathways that induce IFN $\alpha\beta$; the receptor for IFN λ is IL-28R $\alpha\beta$ and is expressed on a limited number of cells, including T cells (Donnelly and Kotenko, 2010). IFN λ signaling into non-T cells diminishes virus replication in target cells and results in an IFN $\alpha\beta$ burst. The effect of IFN λ signaling into antiviral T cells and their ability to form memory cells remains to be investigated. From the standpoint of improving vaccines against viruses, it would be interesting to learn whether the quality or number of memory cells differs when they differentiate with IFN γ signals versus IL-1 β , IFN $\alpha\beta$, IFN λ , IL-12, or IL-18. DNA viruses and RNA viruses differ in the kinds of innate pathways that are stimulated, and there may be consequent effects on virus-specific T cell memory.

TLR ligands also act on T cells to augment their responses (Marsland et al., 2007). MyD88 conveys signals emanating from TLRs that induce IFN within target cells, but there may be additional functions for this molecule. Intriguing data show that MyD88 signals within T cells influence their accumulation after viral infections (Rahman et al., 2008; Zhao et al., 2009; Zhou et al., 2009). Thus, additional MyD88 functions may overlap with MyD88-mediated induction of IFN signaling to enhance antiviral T cell responses. T cell differentiation is likely determined by a variety of signals, including TCR strength and duration,

IFN and other combinations of pro-inflammatory cytokines, and TLR signaling (Nembrini et al., 2006).

Discussion

Antiviral CD4⁺ T cell responses begin from a small number of cells that undergo tremendous rates of cell division and accumulate under the positive influence of inflammatory cytokines. These early signals enhance their number and further their differentiation into memory cells. Once set, CD4 memory is maintained by homeostatic cytokines and by B cells through unknown mechanisms. Th1 memory cells sustain antiviral CD8 T cell responses and are involved in CD8⁺ T cell recruitment to peripheral sites; other lineages of antiviral CD4⁺ T cells can be found after infection, but their longevity and protective role during recall responses need to be studied.

Most studies have examined the influence of innate immune defense on developing T cells, but in an immune individual, the adaptive response occurs concurrently and possibly before innate defenses. How do cytokine-secreting memory T cells affect infected target cells, APC function, and NK cell activity? How do these signals impact ISG functions within infected target cells, given that some proteins (eg, MyD88) may have multiple functions? Conversely, there is evidence that innate immunity influences effective recall responses: in the absence of PKR – a cytoplasmic sensor of viral RNA that stimulates IFN responses – existent memory CD8⁺ T cells did not effectively eliminate a challenge virus infection (Nakayama et al., 2010). Thus, innate detection of infection and the positive effects of inflammatory cytokines on established T cell memory is an important area for future investigation, as it relates to how best to boost immunity with vaccination.

The role of IFNs, costimulatory molecules, and pro-inflammatory cytokines during chronic virus infection could be very different from that seen during acute infection. The immune system is hyper-activated during HIV infection, such continuous stimulation may induce an endogenous program that downregulates adaptive immunity under these circumstances. During chronic virus infection, virus-specific T cells are either deleted or become dysfunctional over time (Brooks et al., 2005; Fuller et al., 2004, 2005; Fuller and Zajac, 2003; Mothe et al., 2007; Zajac et al., 1998a,b). Antiviral CD4⁺ T cells become non-functional like CD8⁺ T cells. The molecular mechanisms underlying these effects are

still being uncovered; current analyses show PD1L–PD1 (Barber et al., 2006; Bengsch et al., 2010; Blackburn et al., 2009; Freeman et al., 2006; Grosso et al., 2009; Wherry et al., 2007), CD27–CD70 (van Gisbergen et al., 2009), CTLA4–B7, Lag3 (Bengsch et al., 2010; Blackburn et al., 2009), and IL10–IL10R (Brooks et al., 2006a,b, 2008; Ejrnaes et al., 2006) interactions inhibit reactive cells during chronic infection in mice and in people. During chronic virus infection, reactive T cells may produce IFN γ – possibly lower amounts than seen during acute infection – and the expression of this cytokine might have inhibitory effects during chronic infection: IFN γ induces PD1L on APCs (Muhlbauer et al., 2006; Schreiner et al., 2004), and IFN γ acts on Treg cells to increase their inhibitory activity. Data in other models indicate that IFN γ signals into non-T cells inhibit memory T cell formation (Sercan et al., 2006, 2010). While the amount and duration of antigen can enhance CD4 T cell responses following acute infection, recent evidence shows that extensive persistence of large amounts of antigen can be detrimental to CD4+ T cell responses (Han et al., 2010), and CD4+ T cells that are removed from such an environment can recover their ability to produce cytokine. Treating chronically infected mice with ribavirin results in a reduction in virus load, and exhausted CD4+ T cells recover some of their functions (Brooks et al., 2006a). Thus, chronic virus infection may diminish CD4+ T cells by induction of immunosuppressive cytokines and inhibitory cell-surface molecules, or by simply over-stimulating them. Future studies need to clarify the roles of type 1 and type 2 IFN on antiviral T cell responses throughout chronic virus infection. Interestingly, persistent LCMV infection of mice does not result in persistently elevated levels of IFN $\alpha\beta$, suggesting that there are other mechanisms at play that diminish innate immune responses—perhaps the inhibited production of IFN $\alpha\beta$ is related to the development of exhausted T cells.

The magnitude of the primary and recall CD4+ T cell response is governed by antigen load and pro-inflammatory cytokines, and the two factors are linked: the antigen load influences how much IFN γ is made by responding T cells and the extent to which they receive direct IFN γ signals. The principal mechanism for priming antiviral CD4+ T cells is through endocytic processes that take-up extracellular protein, degrade it, and load the resulting peptides onto MHCII molecules. However, other mechanisms, including macroautophagy, result in the presentation of peptides from cytosolic proteins (Deretic, 2009; Gannage and Munz, 2009; Lee and Iwasaki, 2008; Munz, 2006, 2007). CD4+ T cell responses form against HSV and EBV (nuclear antigen 1 EBNA1) proteins that are in the cytoplasm of MHCII+ cells. Certain proteins that have escaped ubiquitin-mediated proteasomal degradation can be captured by autophagosomes and transported to lysosomes. These tend to be long-lived proteins and also ones that form large aggregates, which become encapsulated in autophagosomes that fuse with lysosomes, where the proteins are degraded and loaded onto resident MHCII molecules. In addition to macroautophagy, chaperone-mediated autophagy – the active heat-shock protein mediated transport of proteins directly into the lysosome – results in proteolytic degradation of proteins into peptides that bind to MHCII molecules. Virus proteins aggregate to form structures that efficiently package their genomes, which may induce the autophagosomal process and lead to peptides that are recognized by CD4+ T cells. The extent to which macroautophagy contributes to the overall CD4+ T cell response after virus infection remains to be quantified and compared to the well-established exogenous pathway. Type 1 and Type 2 interferons increase the amount of autophagy within cells, which may enhance the amount of antigen is presented to CD4+ T cells and affect the magnitude of the response. There may also be differences in how proteins are degraded in the late endosome/lysosome pathway versus the autophagosome/lysosome pathway, potentially determining the populations of virus-specific CD4+ T cells that respond. Lysosomes that have fused with autophagosomes may contain different sets of molecules compared to lysosomes that fuse with phagosomes; the co-expression of these other molecules with MHCII molecules on the surface of cells might also influence the developing CD4+ T cell response. Many viruses encode proteins that prevent MHC presentation; this may impact CD4+ T cell

responses that depend upon autophagosome-mediated antigen presentation in the same infected cell but not affect CD4+ T cells that recognize antigen produced in the classical phagocytic process or receptor-mediated uptake in non-infected cells. It is unclear what proportion of CD4+ T cells respond to material derived from extracellular protein versus macroautophagy, and how these pathways differ in T cell-dependent protection.

The genomics of antiviral immunity

Nearly all analyses of immune response to vaccination or infection are done in highly inbred mouse models. Pre-clinical studies to evaluate vaccines typically begin with studies in standard inbred mouse models. General response patterns in mice will mimic those in people, but there are exceptions, which need to be studied. Mouse models are useful, because many tools are available to quantify immune responses, which is essential for understanding the underlying processes involved in resolving infection and establishing T cell memory. However, there are several problems with existing mouse models: much of the immune response is dominated by a few genes, different inbred mouse strains have lost some of these genes, and mice vary in their expression of the these genes, which may not match with normal expression patterns. Additionally, many inferences about certain pathways are based on data from gene-ablated mice—such experimental approaches are straightforward and easy to interpret, but rarely do people have absolute deficiencies in those particular genes. Normal individuals vary in alleles of genes and in the amount of expression of those genes, and they differ in non-coding regions of the genome. Critical variation beyond allelic variances at the MHC locus includes differences in intrinsic antiviral pathways and PRRs, including the induction of interferons that amplify differences in subsequent T cell responses. All of these differences with intact genes impact phenotypes seen in people—such natural variation is not replicated in inbred strains of mice. A robust outbred mouse model that factors in the natural variations in components of these networks may better predict which pathways are critical for immunity (Churchill et al., 2004; Yalcin et al., 2010). The quantitative analysis of immune responses following infection or vaccination in outbred populations of mice followed by quantitative associations with certain genetic loci associated with inflammation will play a key role in the future for dissecting how to best develop protective immune responses with vaccines.

Acknowledgment

JKW is supported by AI074862 from the NIH (NIAID).

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